REPORT

A Genomewide Search Finds Major Susceptibility Loci for Nicotine Dependence on Chromosome 10 in African Americans

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Epidemiological studies have demonstrated that genetic factors account for at least 50% of the liability for nicotine dependence (ND). Although several linkage studies have been conducted, all samples to date were primarily of European origin. In this study, we conducted a genomewide scan of 1,261 individuals, representing 402 nuclear families, of African American (AA) origin. We examined 385 autosomal microsatellite markers for ND, which was assessed by smoking quantity (SQ), the Heaviness of Smoking Index (HSI), and the Fagerström Test for ND (FTND). After performing linkage analyses using various methods implemented in the GENEHUNTER and S.A.G.E. programs, we found a region near marker *D10S1432* on chromosome 10q22 that showed a significant linkage to indexed SQ, with a maximum LOD score of 4.17 at 92 cM and suggestive linkage to HSI, SQ, and log-transformed SQ. Additionally, we identified three regions that met the criteria for suggestive linkage to at least one ND measure: on chromosomes 9q31 at marker *D9S1825,* 11p11 between markers *D11S1993* and *D11S1344,* and 13q13 between markers *D13S325* and *D13S788*. Other locations on chromosomes 15p11, 17q25, and 18q12 exhibited some evidence of linkage for ND (LOD >1.44). The four regions with significant or suggestive linkage were positive for multiple ND measures by multiple statistical methods. Some of these regions have been linked to smoking behavior at nominally significant levels in other studies, which provides independent replication of the regions for ND in different cohorts. In summary, we found significant linkage on chromosome 10q22 and suggestive linkage on chromosomes 9, 11, and 13 for major genetic determinants of ND in an AA sample. Further analysis of these positive regions by fine mapping and/or association analysis is thus warranted. To our knowledge, this study represents the first genomewide linkage scan of ND in an AA sample.

Tobacco smoking is a major world health problem, and nearly one-third of the global adult population smokes tobacco products.[1] Nicotine dependence (ND [MIM 188890]), like many other substance-dependence disorders, is a complex trait determined strongly by both genetic and environmental factors.[2,3] Our recent meta-analysis of reported twin studies indicated that genetic factors account for at least 50% of the liability for this complex trait.[3] Thus, identifying the genes that predispose one to ND and understanding its molecular mechanisms is vital to prevention and treatment.

At least 10 studies have mapped susceptibility loci for ND through use of genomewide linkage analysis of smoking behavior.[4–13] Although numerous regions have been identified as susceptibility loci for ND, most have not been replicated, except for those on chromosomes 9q22 and 11p11, which have received broad support.[5,14] Furthermore, almost all participants in those studies were primarily of European origin. Because there are known differences in smoking patterns and in ND risk across ethnic groups, which may reflect different risk loci,[15–18] it is important to conduct linkage analysis across populations. The primary purpose of the present study was to identify susceptibility loci for ND in an African American (AA) sample.

Participants were of AA origin from the Mid-South Tobacco Family (MSTF) cohort, recruited primarily from the states of Tennessee, Mississippi, and Arkansas during 1999–2004. Proband smokers were required to be at least 21 years old, to have smoked for at least the past 5 years, and to have consumed an average of 20 cigarettes per day for the past 12 mo. Smoking participants were assessed for ND with the Fagerström Test for Nicotine Dependence.[19] Once a proband and a full sibling who was also nicotine dependent (for most of our families) were identified, additional siblings and biological parents were recruited whenever possible, regardless of their smoking status. In the case where biological parents of a proband were not available, we attempted to recruit at least three of the proband's full siblings. A total of 1,261 subjects from 402 AA families were included in the study. Given the recruitment criteria employed in the study, nonsmokers could not be considered nicotine naive and were included in the linkage analyses. The family size had a range of 2–9, and the

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Table 1. Demographic and Pedigree Characteristics of the Data Set

Characteristic	Value
No. of families	402
Mean ± SD no. of family members (range)	3.14 ± 0.78 (2–9)
No. of genotyped subjects	1,261
Mean ± SD age (in years)	40.07 ± 14.56
Percentage female	66.1
No. of smokers	1,013
Mean ± SD age at smoking onset (in years)	17.36 ± 4.79
Mean ± SD no. of smoking years	21.06 ± 12.52
No. ± SD of cigarettes per d	22.16 ± 11.82
Mean ± SD HSI score	3.74 ± 1.49
Mean ± SD FTND score	6.27 ± 2.17
No. of study families with:	
0 Parent included in the study	187
1 Parent included in the study	199
2 Parents included in the study	16
No. of study families with:	
1 Smoker	31
2 Smokers	210
⩾3 Smokers	161
No. of study families with:	
1 Full sibling	22
2 Full siblings	174
⩾3 Full siblings	206
No. of study pairs of:	
Parent and offspring	512
Sib pairs	926
Sisters	405
Brothers	180
Brother and sister	341

average size (±SD) was 3.14 ± 0.78. Of these families, 53.5% had at least one biological parent recruited, 43.3% had two full siblings, and 51.2% had three or more full siblings. Among the participants of the study, the majority (smokers 69.4%; nonsmokers 65.7%) had a high school education or less, and most of them were from families considered low income. Descriptive statistics regarding demographic and clinical characteristics of the sample are presented in table 1. All participants provided informed consent, and the study was approved by all involved institutional review boards.

Several measures were used to assess ND, including the number of cigarettes smoked per day,[20,21] current tobacco use,[22] and Fagerström Test for ND (FTND) score.[23,24] Although these measures are not identical, they are highly correlated.[2,3] In the present study, the ND of each smoker was ascertained by the three measures most commonly used in the literature: smoking quantity (SQ), defined as the number of cigarettes smoked per day; the Heaviness of Smoking Index (HSI), a 0–6 scale, which includes SQ and smoking urgency (i.e., how soon after waking the subject smokes the first cigarette; these are questions 1 and 3 of the FTND); and the FTND score, a 0–10 scale.[19] The correlation coefficients among the three ND measures had a range of 0.88–0.94 in our sample. After considering the effect of abnormal distribution of the phenotype on our linkage results, as we did previously,[11] we transformed nonzero SQ value to a natural log-scale or an indexed SQ, in which individuals who smoked 1–10, 11–20, 21–30, and ⩾31 cigarettes per d were assigned the values 0, 1, 2, and 3, respectively, per the FTND item coding. Skewness and kurtosis values, respectively, were 1.20 and 2.69 for the SQ, −0.80 and 1.19 for log-transformed SQ, 0.41 and −0.75 for indexed SQ, −0.46 and −0.15 for HSI, and −0.55 and −0.40 for the FTND.

DNA was extracted from peripheral-blood samples of each participant. Genotyping for microsatellite markers was performed at the Center for Inherited Disease Research (CIDR) at Johns Hopkins University. The CIDR marker set was composed primarily of trinucleotide and tetranucleotide repeats and consisted of 404 markers (385 on autosomal chromosomes, 16 on chromosome X, and 3 on chromosome Y), with average spacing of 8.6 cM

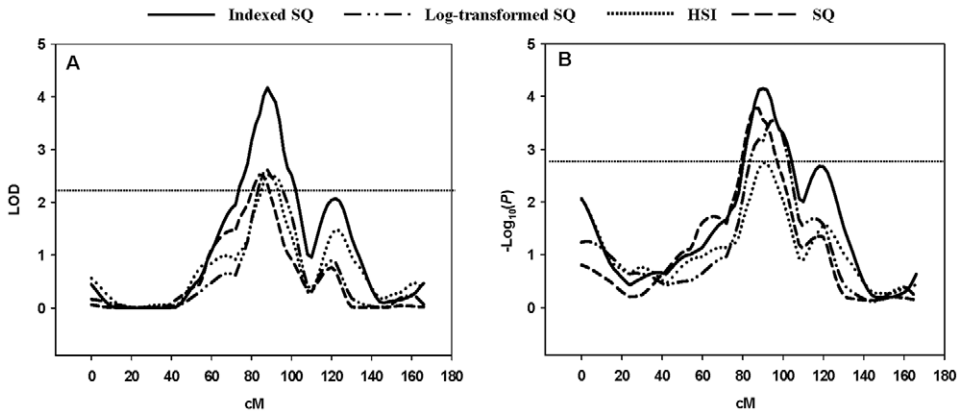

Figure 1. Multipoint linkage–analysis results from the EM-HE regression method of GENEHUNTER (*A*) and the SIBPAL program of S.A.G.E. (*B*) for indexed SQ, SQ, HSI, and log-transformed SQ on chromosome 10. Genetic distance (in cM) is plotted on the *X*-axis against the LOD score from GENEHUNTER (*A*) and against $-\log_{10}(P)$ from SIBPAL (*B*). The horizontal line in each graph corresponds to the threshold for suggestive linkage.[28,38]

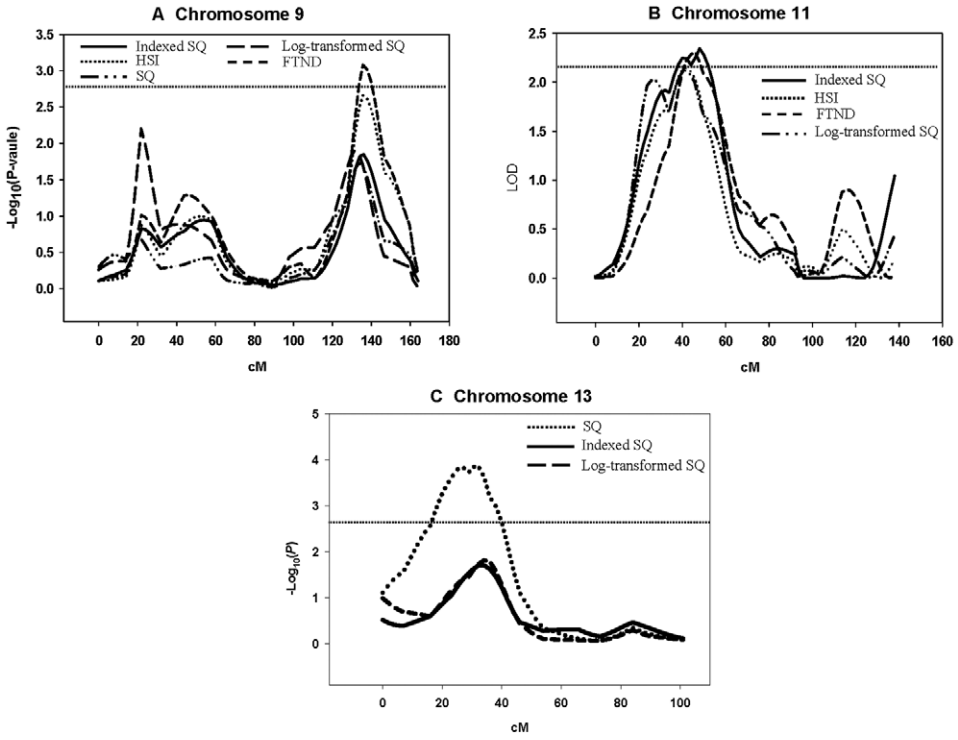

Figure 2. Linkage-analysis results of various ND measures on chromosomes 9, 11, and 13. Genetic distance (in cM) is plotted on the *X*-axis against $-\log_{10}(P)$ from SIBPAL for panels A and C and against the LOD score from GENEHUNTER for panel B. The horizontal line in each graph corresponds to the threshold for suggestive linkage.[28,38]

throughout the genome. There were no gaps in the map that were >18 cM, and the average marker heterozygosity was 0.76. Only 385 autosomal markers were used in the present study. Extensive quality checks were performed to verify the consistency of marker genotyping and of stated pedigree relationships. The PedCheck program was used to identify any inconsistent Mendelian inheritance, nonpaternity, or typing errors.[25] The errors (a total of 7,971 Mendelian inconsistencies, an ~1.1% error rate) identified in PedCheck were assumed to have occurred in the genotyping process, and the associated markers were set to "missing" for the corresponding members. Also, we used all markers to check pedigree errors by means of the RelCheck program.[26] To avoid bias, the individuals with a false genetic (blood) relationship were excluded from further linkage analysis.

Two linkage analysis programs (SIBPAL in S.A.G.E. v. 5.1 and GENEHUNTER v. 2.1) were used. For the GENE-

Table 2. Chromosomal Regions Potentially Linked to Different ND Measures

Chromosome, Marker(s) (Distance, in cM, from p-ter), and ND Measure	Marker at or Near Peak	Peak Position (cM)	Chromosome Location	Nominal *P*	Maximum LOD Score	Program Used for Linkage Analysis
Chromosome 15:						
D15S128-D15S165 (0–20):						
FTND	*D15S128*	6	15p11.1	2.2×10^{-3}		SIBPAL
HSI	*D15S128*	6	15p11.1	8.6×10^{-3}		SIBPAL
Indexed SQ	*D15S128*	6	15p11.1	1.7×10^{-2}		SIBPAL
Chromosome 17:						
D17S2193-D17S928 (89–126):						
Indexed SQ	*D17S784*	117	17q25.3		1.99	Nonparametric (GENEHUNTER)
SQ	*D17S784*	117	17q25.3		1.68	Nonparametric
HSI	*D17S784*	117	17q25.3		1.34	Nonparametric
Chromosome 18:						
D18S843-D18S1364 (28–100):						
FTND	*D18S535*	67	18q12.1-18q12.2	2.0×10^{-3}		SIBPAL
HSI	*D18S535*	69	18q12.1-18q12.2	7.5×10^{-3}		SIBPAL
Indexed SQ	*D18S535*	69	18q12.1-18q12.2	1.2×10^{-2}		SIBPAL

The figure is available in its entirety in the online edition of *The American Journal of Human Genetics*.

Figure 3. Multipoint linkage–analysis results from the SIBPAL program of S.A.G.E. The legend is available in its entirety in the online edition of *The American Journal of Human Genetics*.

HUNTER program, we used the expectation-maximization (EM)–Haseman-Elston (HE) regression and nonparametric method to analyze all ND measures. Multipoint methods were used for estimating identity-by-descent (IBD) sharing needed for calculating these statistics. Other conditions for running the programs used the relevant program defaults. For S.A.G.E., we used genotypes from all family members to calculate multipoint IBD allele-sharing distributions with the GENIBD program. SIBPAL, a model-free S.A.G.E. linkage program, was then used to perform the linkage analysis on the basis of all possible sib pairs. Evidence of linkage was assessed by an HE regression, with dependent-variable options W3 and W4, both of which transform the sib pair's trait values to weighted combinations of the squared trait difference and squared mean corrected trait sum, but the former option adjusted for the nonindependence of sib pairs, whereas the latter option adjusted for the nonindependence of sib pairs and the nonindependence of squared trait sums and differences.[27] Otherwise, default options were used for all parameters in the trait regression method. Sex and age were included as covariates for all analyses reported in this communication. To determine empirical estimates of genomewide significance level, we performed linkage analysis on 1,000 replicate permutations, using the approach we described elsewhere,[12] with both the family structure and the IBD structure kept intact. The empirical significance level of an observed LOD score or *P* value for an ND measure of interest was then estimated by counting the proportion of genome scans containing one or more peaks of that value. The significant linkage threshold was defined as the LOD score or *P* value occurring in 50 of the 1,000 permutations, corresponding to a probability of 0.05 in a genome scan.[28] The S-Plus 6.1 and SAS 8.2 packages were used to prepare the required data format for the linkage-analysis programs and to summarize the data generated from the programs.

Multipoint linkage analyses revealed significant or suggestive evidence of linkage on four chromosomes (figs. 1 and 2), on the basis of the genomewide significance threshold of Lander and Kruglyak.[28] As shown in figure 1, a single region on chromosome 10q22 showed significant evidence of linkage to indexed SQ between markers *D10S1208* and *D10S2470*. This linkage was detected by both the EM-HE method of GENEHUNTER, with a LOD score of 4.17 at 92 cM (the empirical threshold LOD score at the genomewide .05 significance level for indexed SQ is 3.50), and SIBPAL, with multipoint nominal $P = .000060$ at 94 cM (W3 and W4 options yielded essentially the same results on the ND measures: close to the empirical *P* value of 5.98×10^{-5}, with an α level of .05). The evidence of linkage of the region on chromosome 10q22 to ND was further supported by the linkage results from analysis of three other ND measures—namely, SQ, log-transformed SQ, and HSI—with use of both linkage programs (fig. 1*A* and 1*B*).

We found three additional regions, located on chromosomes 9, 11, and 13, that showed suggestive linkage to at least one of the ND measures (fig. 2*A*–2*C*). For example, we found suggestive evidence of linkage to indexed SQ and FTND near marker *D11S1344* on chromosome 11p11, with LOD scores of 2.35 and 2.31, respectively (fig. 2*B*). In the same region, between markers *D11S1392* and *D11S1344* (~43–58 cM), we found evidence of potential linkage to HSI, with a LOD score of 2.15 at 51 cM. On chromosome 9q31 at marker *D9S1825,* we found suggestive linkage to FTND with a multipoint nominal *P* value of .00083 and potential linkage to HSI ($P = .0021$) at a distance of 136 cM from the p telomere (fig. 2*A*). Additionally, we found suggestive linkage of SQ to chromosome 13q13 between markers *D13S325* and *D13S788,* with multipoint nominal $P = .000135$ at 42 cM (fig. 2*C*).

Three other regions showed a multipoint nominal *P* value or LOD score that was just below the threshold for suggestive linkage. These include the region on chromosomes 15p11 at marker *D15S128,* 17q25 at marker *D17S784,* and 18q12 between markers *D18S535* and *D18S851* (table 2). Of these potential linkages, we found that FTND showed a peak on chromosome 15 at 6 cM and a peak on chromosome 18 at 67 cM, with multipoint nominal *P* values of .0022 and .002, respectively. Similarly, we found evidence of a potential linkage of indexed SQ to chromosome 17 at 117 cM, with a LOD score of 1.99. Such a potential linkage to these three regions has been supported by the other ND measures used in this study (see table 2 for details).

The strong statistical support for linkage of ND at 10q22.1-10q22.2 is novel and highly significant. In a previous genome scan, a weak linkage signal for ND (assessed by the FTND) was observed for a region that overlapped slightly with the region identified herein, with a *P* value of .0004 at 127.1 cM near marker *D10S2469,* in the Christchurch, New Zealand,[29] sample. Given the fact that the subjects of that study were primarily of European origin,

The figure is available in its entirety in the online edition of *The American Journal of Human Genetics*.

Figure 4. Multipoint linkage–analysis results from the nonparametric method of GENEHUNTER. The legend is available in its entirety in the online edition of *The American Journal of Human Genetics*.

The figure is available in its entirety in the online edition of *The American Journal of Human Genetics*.

Figure 5. Multipoint linkage–analysis results from EM-HE regression method of GENEHUNTER. The legend is available in its entirety in the online edition of *The American Journal of Human Genetics*.

whereas those in the present study were of AA origin, this region appears to be a potential susceptibility locus for ND in both ethnic groups, with likely smaller effects for European compared with AA smokers. On the basis of the human genome sequence from public databases (Map Viewer), there are ~400 genes within this region. Of these, the genes ionotropic glutamate receptor delta 1 (*GRID1*), dynamin binding protein, and catechol-O-methyltransferase domain containing 1 represent plausible candidates for an association study, on the basis of their biological function(s) and the biochemical pathways involved in drug addiction.[30–32] However, there is no reported study demonstrating an association of these candidate genes with ND or with other psychiatric disorders, with the exception that *GRID1* has been associated with bipolar disorder and schizophrenia.[32]

Of the linked regions identified in this study, the susceptibility loci on chromosomes 9 and 11 have been reported in multiple studies. Elsewhere, we identified an ~13-cM interval on chromosome 9q22 that showed suggestive linkage to ND in Framingham Heart Study (FHS) families.[11] Linkage of this region to smoking behavior at a nominally significant level has been supported by three other independent studies.[7,9,10] Several positional candidate genes, such as $GABA_B$ receptor subunit 2 (*GABAB2* [MIM 607340]) and neurotrophic tyrosine kinase receptor type 2 (*NTRK2* [MIM 600456]), within the 9q22 linkage region were found to be associated with ND in our recent family-based association study of the MSTF sample.[33,34] The region on chromosome 11q12 is another susceptibility locus for ND that has been reported in multiple studies.[4,9,11,35] Of these studies, we have reported the strongest linkage results for smoking consumption on chromosome 11q12, with a LOD score of 3.95 in the FHS sample.[11] This linkage has been replicated at a nominal level by Goode et al.,[35] with a LOD score of 1.10 for cigarette consumption in the same cohort. Recently, Morley et al.[4] reported a suggestive linkage of cigarette consumption to chromosome 11q13 ($P = .0000568$) in an Australian twin sample. In another study, likely linkage on chromosome 11q14 for smoking behavior was reported, with a LOD score of 1.64.[9] Our finding of suggestive linkage to ND on chromosome 11 is further supported by a significant association with brain-derived neurotropic factor (*BDNF* [MIM 113505]), located within the region at 11p14.1 near the linkage peak shown in figure 2*B*.[36]

Another region showing suggestive linkage to ND is located on chromosome 13q12, with a multipoint nominal P value of 1.35×10^{-4}. Previously, this region was weakly linked to smoking behavior in two independent studies, with a LOD score of 1.3 for both.[13,37] However, because we detected suggestive linkage of the region on chromosome 13 to SQ only, more replication studies are needed to determine whether this linkage was attributable to chance or is derived from loci with small effects. Further, we identified potential linkage of chromosomes 15, 17, and 18 to ND. Although these three regions do not meet the threshold for suggestive linkage to ND,[28] they potentially harbor susceptibility genes for ND, since all these regions have been linked to smoking behavior at a nominally significant level in independent samples. For example, Bierut et al.[9] identified a linkage of habitual smoking to chromosome 15 at 14 cM, with a LOD score of 1.25, in the sample from the Collaborative Study on the Genetics of Alcoholism (COGA). Using the same COGA data set, Bergen et al.[7] reported potential linkage to chromosome 17 at ~111 cM for ever-smoking versus never-smoking status and to chromosome 18 at 18.7 cM for number of cigarette packs per year, with P values of .0001 and .0002, respectively.

In conclusion, we provide strong evidence of significant linkage of ND to chromosome 10 and suggestive linkages to chromosomes 9, 11, and 13 in an AA sample. Further, we identified three regions on chromosomes 15, 17, and 18 that showed potential linkage to ND. There is some overlap between our results and those of previous genome scans that examined smoking behavior in other ethnic samples. To our knowledge, our study represents the first genomewide scan for ND in an AA population. Given the potential ethnic differences in smoking patterns and nicotine metabolism, this study may shed light on strategies for searching for susceptibility loci/genes for ND, especially in AA smokers. These regions represent excellent candidate loci that harbor allelic variants that alter vulnerability to ND. Further analysis of these regions by fine mapping or association analysis is thus warranted.

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Web Resources

The URLs for data presented herein are as follows:

CIDR, http://www.cidr.jhmi.edu/
Map Viewer, http://www.ncbi.nlm.nih.gov/mapview/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for ND, *GABAB2, NTRK2,* and *BDNF*)
S.A.G.E., http://darwin.cwru.edu/sage/

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